



# Effect of water stress “memory” on plant behavior during subsequent drought stress

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## ABSTRACT

Frequency of extreme drought events are expected to increase due to climate change. Perennials are increasingly exposed to recurrent drought during their life span. The aim of the present work was to study the effect of recurrent droughts on the behavior of *Vitis vinifera* under water stress. Sangiovese and Montepulciano vines were exposed to severe drought stress for 4 years (WS-S). A dry-down experiment was carried out to compare their behavior with a set of vines kept at 90% field capacity during the whole seasons in the previous 4 years (WW-S). WS-S vines had higher transpiration and stomatal conductance than WW-S vines. Net photosynthesis was almost unaffected by the treatment. Stomatal conductance was higher at more negative  $\Psi_{\text{stem}}$  in WS-S vines than in control vines. Leaf petiole percentage loss of hydraulic conductance, measured during water stress, was higher in WS-S than in WW-S vines. Results indicate that previous water stress can lead to less conservative plant strategy toward water loss and decreased water use efficiency. This behavior seems to be coordinated with the different stomatal response to decreasing water potential that caused a reduction of xylem hydraulic safety margin in WS-S vines in comparison with WW-S vines.

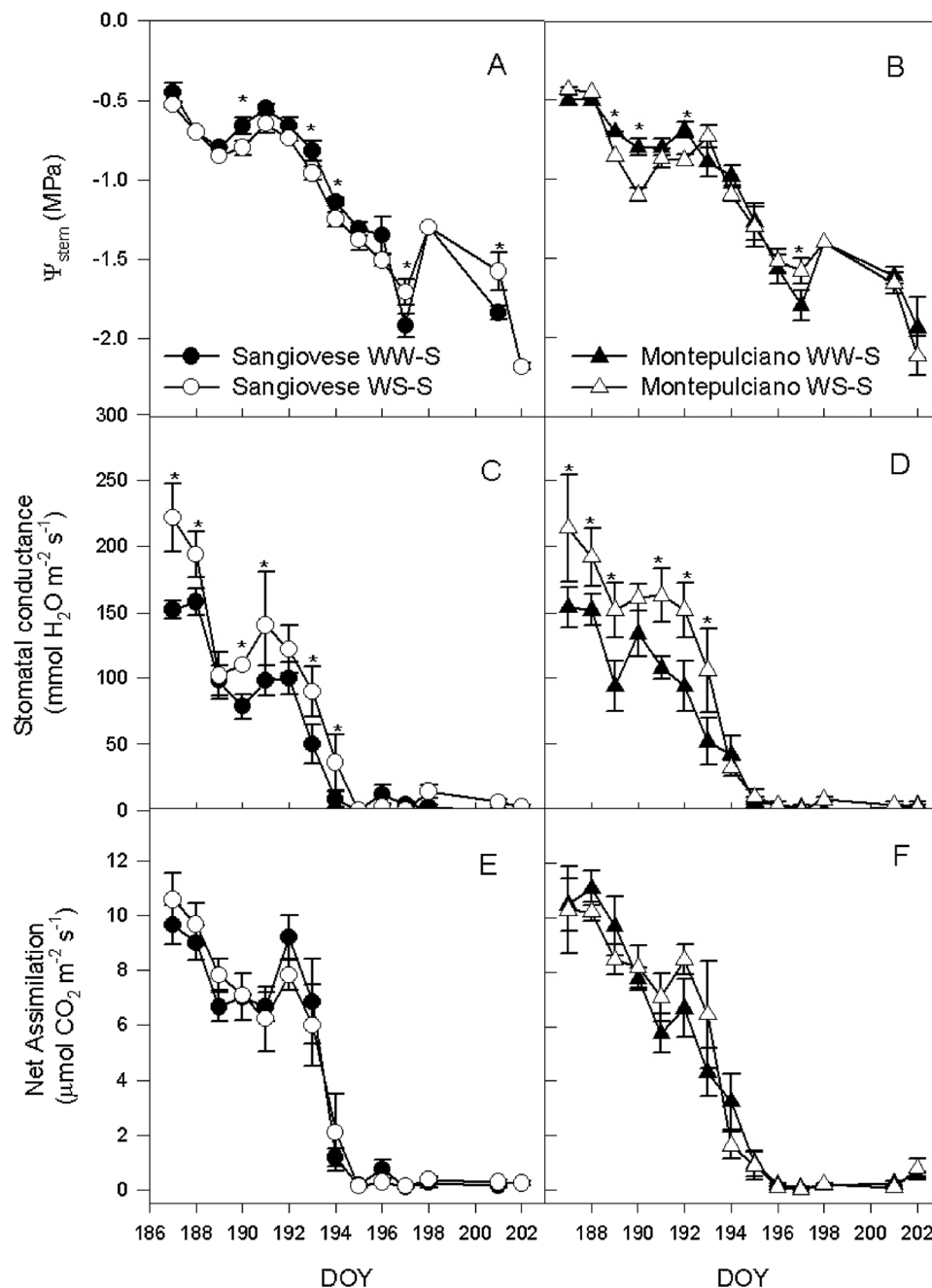
## 1. Introduction

Drought event frequency and severity are expected to increase in the near future as result of the decrease of regional precipitation and the increase in evapotranspiration driven by global warming (Sheffield and Wood, 2008; Sheffield et al., 2012; Dai, 2010). Changes in the global water cycle in response to the warming over the 21st century will not be uniform, and natural droughts are expected to set in quicker, to become more intense, and to last longer (Trenberth et al., 2014). Among all the natural hazards, drought ranks first in terms of the number of people directly affected (Wilhite, 2000). Taking into account agriculture, drought, under vulnerable situations, can endanger food security with cascade effects on economy, geopolitics and society (Grayson, 2013). Thus, the understanding of how plants and crops adapt and behave under this scenario is a goal of primary importance to improve agricultural performance towards recurrent droughts and to address the challenges of climate change, consequently.

Drought endangers the survival of both annual and perennial species, though annual species have a larger number of options to withstand and adapt to climate change than perennial species. While annual species can change their phenotype according to changing climatic condition by evolution or plasticity, in perennials, and in particular in

tree species, climate change adaptation by evolution is largely unavailable due to their relatively long reproduction cycle (Franks et al., 2014). Thus, considering forest species, the fate of tree species in changing environment is mainly to persist through migration, or to adapt through phenotype plasticity, or to disappear (Aitken et al., 2008). Focusing on agricultural tree crops, there are many socio-economic factors (i.e. long re-establishment periods, closeness to processing plants, availability of skilled hand labor, and accessible markets) that make production area change difficult (Glenn et al., 2014). For instance in grapevine (*Vitis vinifera* L.), the most important tree crop in the world in terms of value (FAOSTAT, 2018), the temperature and water deficit increases in the next centuries are projected to make Mediterranean basin (currently the most important cultivation area in the world) progressively unsuitable for cultivation with consistent socioeconomic impact on that region (Moriondo et al., 2013). *V. vinifera* is also one of the most important model species for studying drought effect in tree species (Lovisolo et al., 2010), and a large number of studies describe the effect of water deficit on grapevine physiology and on the ability of recovery after drought (for a review see Chaves et al., 2010 and Lovisolo et al., 2010). When water stress occurs, plant growth and overall carbon fixation are reduced with significant negative consequences on plant productivity and, in extreme cases, on plant survival

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**Fig. 1.** Trend of stem water potential (A, B), Stomatal conductance (C, D) and Net assimilation (E, F) in Control and WS vines of Sangiovese (A, C, E) and Montepulciano (B, D, F) during the experiment (DOY, days of the year). Points marked with \* are different per  $P = 0.05$  (t test).

(Pallioti et al., 2014). When water availability in the soil becomes critical for vines, stomatal closure acts as an early response, buffering the drop of xylem water potential and the consequent risk of massive xylem embolism and catastrophic hydraulic failure (Jones and Sutherland, 1991; Tyree and Sperry, 1989). The onset of stomatal closure coincides with the reaching of critical tension (i.e. water potential) in the stem xylem (Salleo et al., 2000; Choat et al., 2012). Stomatal functionality appears to be regulated primarily by hydraulic signals (Nardini and Salleo, 2000; Franks, 2004), although, under water stress conditions, stomata responds also other to chemical or hormonal signals produced by the dehydrating roots. The most important hormonal signal in this regard is abscisic acid (ABA), which has been very well observed in dehydrating roots and circulating in plants under water deficit conditions (Chaves et al., 2002). ABA is thought to produce its effect through its interaction with other chemicals like jasmonic acid

(JA), cytokinins, auxin and ethylene (Tuteja and Sopory, 2008). They produce their effect through ion exchange, cytoskeletal reorganization, metabolite production, modulation of gene expression and post-translational modification of proteins (Sarwat and Tuteja, 2017). ABA and JA are positive regulators of stomatal closure, auxins and cytokinins are negative regulators and the role of ethylene dependent on the tissue and its condition (Daszkowska-Golec and Szarejko, 2013).

Drought effects (in particular water stress) on the physiological behavior of annual and perennial species have been widely investigated. Nevertheless, scant information is available on the behavior of perennial species subjected to recurrent drought. Trees in areas vulnerable to drought are exposed to drought stress almost every year, and this could naturally prime tree response to drought. In fact, plant stress ‘memory’, i.e. the structural, genetic, and biochemical modifications that have occurred because of stress exposure and which make

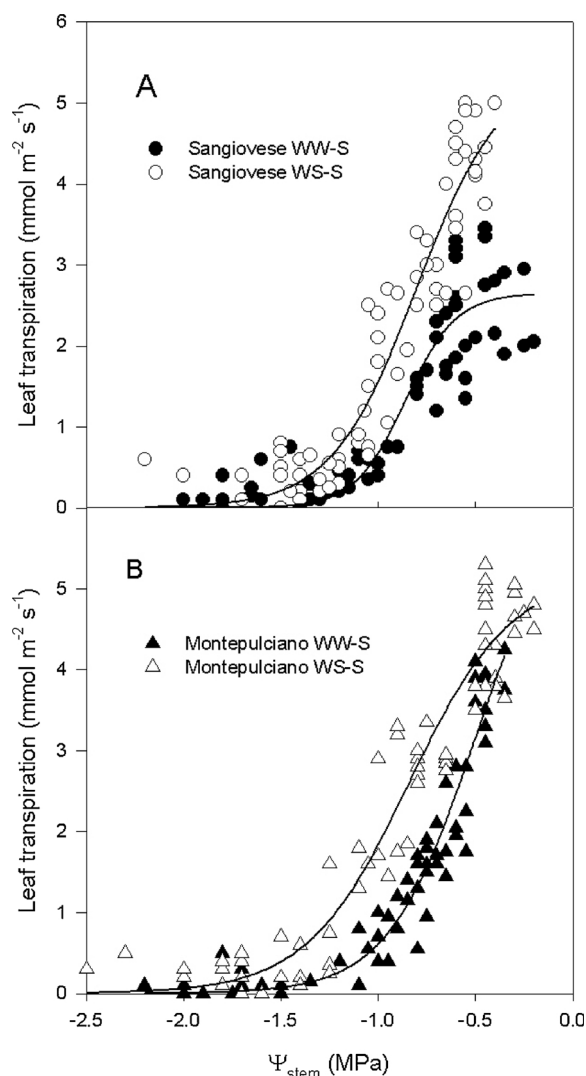


Fig. 2. Leaf transpiration vs. Stem water potential in Control and WS vines of Sangiovese (WW-S  $y = 2.65/(1 + \exp(-(x + 0.86)/0.13))$ ,  $R^2 = 0.92$   $P < 0.0001$ ; WS-S  $y = 5.43/(1 + \exp(-(x + 0.80)/0.22))$ ,  $R^2 = 0.92$   $P < 0.0001$ ) (A) and Montepulciano (B) (WW-S  $y = 6.29/(1 + \exp(-(x + 0.50)/0.22))$ ,  $R^2 = 0.93$   $P < 0.0001$ ; WS-S  $y = 5.20/(1 + \exp(-(x + 0.84)/0.26))$ ,  $R^2 = 0.93$   $P < 0.0001$ ).

plant more resistant to future exposure to the same stress factor, is gaining rising attention (Fleta-Soriano and Munné-Bosch, 2016). It is known that water stress conditions during the vegetative season can induce morpho-anatomical changes that have been hypothesized to increase tolerance towards water stress (Lovisolo and Schubert, 1998; Palliotti et al., 2014); on the other hand, water stress appears to increase plant susceptibility to drought because of increased xylem vulnerability due to phenomena such as cavitation fatigue and carbohydrate depletion (Hacke et al., 2001; Trifilò et al., 2017). Currently there is a lack of knowledge about the possible contrasting behavior of perennials grown under recurrent droughts vs. optimal water availability. In fact, plants used in water stress experiments are subjected to short cycles of water stress (anywhere between 1 week and one vegetative season), and experiments about plant priming or multiple cycle of drought rarely encompass more than one season (Fleta-Soriano and Munné-Bosch, 2016).

We hypothesized that repeated drought stress during perennial plant life span can affect plant water relations, by modifying their water use in relation to the carbon gain. In particular we hypothesized an increase in petiole percent loss of hydraulic conductivity (PLC) and a more conservative regulation of stomata dynamics in plants exposed to

water stress in comparison with non-previously stressed plants.

The aim of this work was to determine if recurrent drought over multiple years can improve plant resistance to drought. More specifically, we aimed to test, on plant subjected to recurrent drought and plant exposed to optimal water availability, 1) the regulation of water loss and carbon fixation under water stress of increasing severity during a dry-down experiment, 2) the link, in these particular plants, between xylem vulnerability and stomatal regulation.

## 2. Materials and methods

### 2.1. Plant material

The study was conducted during July 2013 on 8-year-old potted *V. vinifera* vines of two top-grown red Italian cultivars i.e. Sangiovese (clone VCR30) and Montepulciano (clone R7), both grafted onto 1103 Paulsen rootstock and grown in an outdoor area in full sun close to the Department of Agricultural, Food and Environmental Sciences of the University of Perugia (Region of Umbria, central Italy, 42°58'N, 12°24'E, elevation 405 m a.s.l.). Pots (60 l volume) were filled with loam soil. At the end of February, each vine was pruned to retain four spurs with two buds each. Pots were painted with white paint every year in the springtime to prevent pot temperature increase. All shoots were oriented upright using suitable stakes. Plants were healthy, had a vegetative vigour consistent with that of similar plants in the field (canes length ~1.8–2 m), borne about 1–2 clusters per cane of a size normal according to the two cultivar standard. A total number of forty vines was used in the experiment. In each cultivar, vines were split into two treatments: WW-S vines were well irrigated (at 90% of field capacity) over all the seasons in the previous 4 years; WS-S vines underwent a water stress (40% field capacity  $\Psi_{stem}$  at midday  $-1.3$  MPa) between fruit set (early June) and veraison (early August) in the previous 4 years. In 2013, vines were initially maintained at field capacity until 7th July. Water was supplied every day at 8:00 pm. On 8th July 2013, drought was imposed on all vines by completely suspending irrigation and covering pot surface by white plastic film. The drought treatment was continued until complete leaf abscission in both cultivars.

### 2.2. Gas exchange and water potential

Stomatal conductance ( $g_s$ ) and net assimilation ( $A_n$ ) measurements were carried out on adult primary leaves grown between the 4th and the 10th node from the shoot base. Measurements were carried out daily between 12:00 am and 1:00 pm, 4:00 am and 5:00 am, 8:00 am and 9:00 am, 4:00 pm and 5:00 pm (for simplicity only measures carried out at midday are reported) from 8th until 23rd July. One representative leaf per vine, sampled from 5 vines (per cultivar) and treatment, was used for gas exchange measurements using an open gas exchange system (ADC-System, LCA-3, Hoddesdon, UK) equipped with a Parkinson leaf chamber (11.2 cm<sup>2</sup>). Daytime measurements (8:00 am to 5 pm) were performed under saturating light conditions (PPFD > 1200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Stem water potential ( $\Psi_{stem}$ ) was measured over the same days and daytimes using a pressure chamber (Soilmoisture Corp, Santa Barbara, CA, USA).  $\Psi_{stem}$  was measured on each vine on one mature leaf that had been wrapped in a plastic film and aluminum foil 2 h prior to the measurements (McCutchan and Shackel, 1992). Water use efficiency was calculated as the ratio between net carbon assimilation ( $A_n$ ) and leaf transpiration (E), excluding measurements with  $A_n$  equal or below 0.

### 2.3. Percentage loss of hydraulic conductance

Percentage loss of xylem hydraulic conductance (PLC) was measured on five petioles per cultivar harvested between 12 a.m. and 1 p.m. on each day during the dry-down experiment. Petioles were cut under

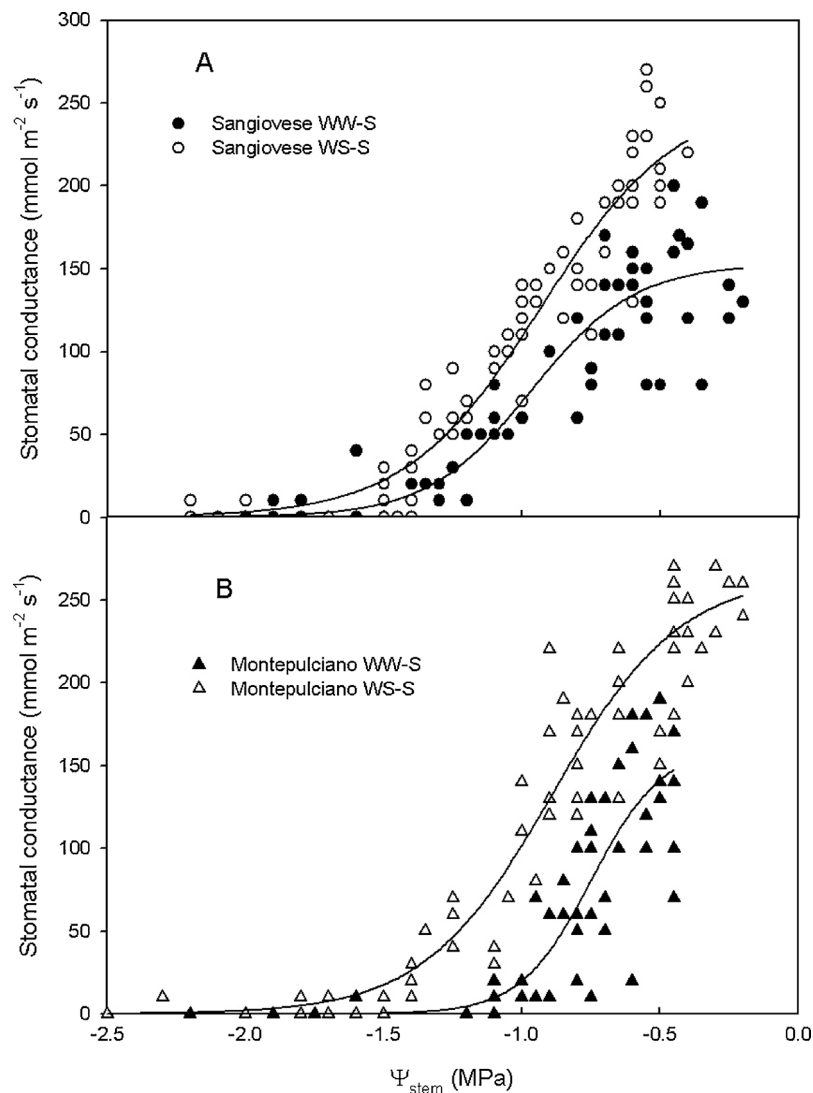


Fig. 3. Stomatal conductance vs. Stem water potential in Control and WS vines of Sangiovese (WW-S  $y = 0.15/(1 + \exp(-(x + 0.96)/0.19))$ ,  $R^2 = 0.83$   $P < 0.0001$ ; WS-S  $y = 0.25/(1 + \exp(-(x + 0.93)/0.24))$ ,  $R^2 = 0.92$   $P < 0.0001$ ) (A) and Montepulciano (B) (WW-S  $y = 0.16/(1 + \exp(-(x + 0.74)/0.13))$ ,  $R^2 = 0.79$   $P < 0.0001$ ; WS-S  $y = 0.26/(1 + \exp(-(x + 0.89)/0.23))$ ,  $R^2 = 0.92$   $P < 0.0001$ ).

water from leaves inserted nearby those used for gas exchange measurements. Hydraulic conductance of petioles was measured by connecting one sample end to plastic tubing filled with a filtered (0.2 μm) 20 mM KCl solution and connected to a pressure head maintained at a pressure (P) of 6 kPa. Flow (F) was measured by collecting the fluid from the distal end in pre-weighed sponge pieces fitted in plastic tubes. Flow readings were taken over 1 min time intervals. After approximately 30 min, once flow was found to be steady, sample hydraulic conductance (K) was calculated as  $F/P$ , and the samples were flushed at  $P = 0.2$  MPa for 30 min, to remove eventual embolism. After flushing samples, maximum hydraulic conductance ( $K_{max}$ ) was measured as above.

Percentage loss of hydraulic conductance of the petiole (PLC) was calculated as:

$$PLC_{petiole} = 100 \times (K_{max} - K)/K_{max}$$

Plant hydraulic conductance was calculated according to (Sperry and Pockman, 1993) as:

$$K_{plant} = E/(\Psi_{pd} - \Psi_{leaf})$$

where E was the leaf transpiration,  $\Psi_{pd}$  was predawn water potential and  $\Psi_{leaf}$  was leaf water potential. Percentage loss of Plant hydraulic

conductance (PLC<sub>plant</sub>) was calculated as:

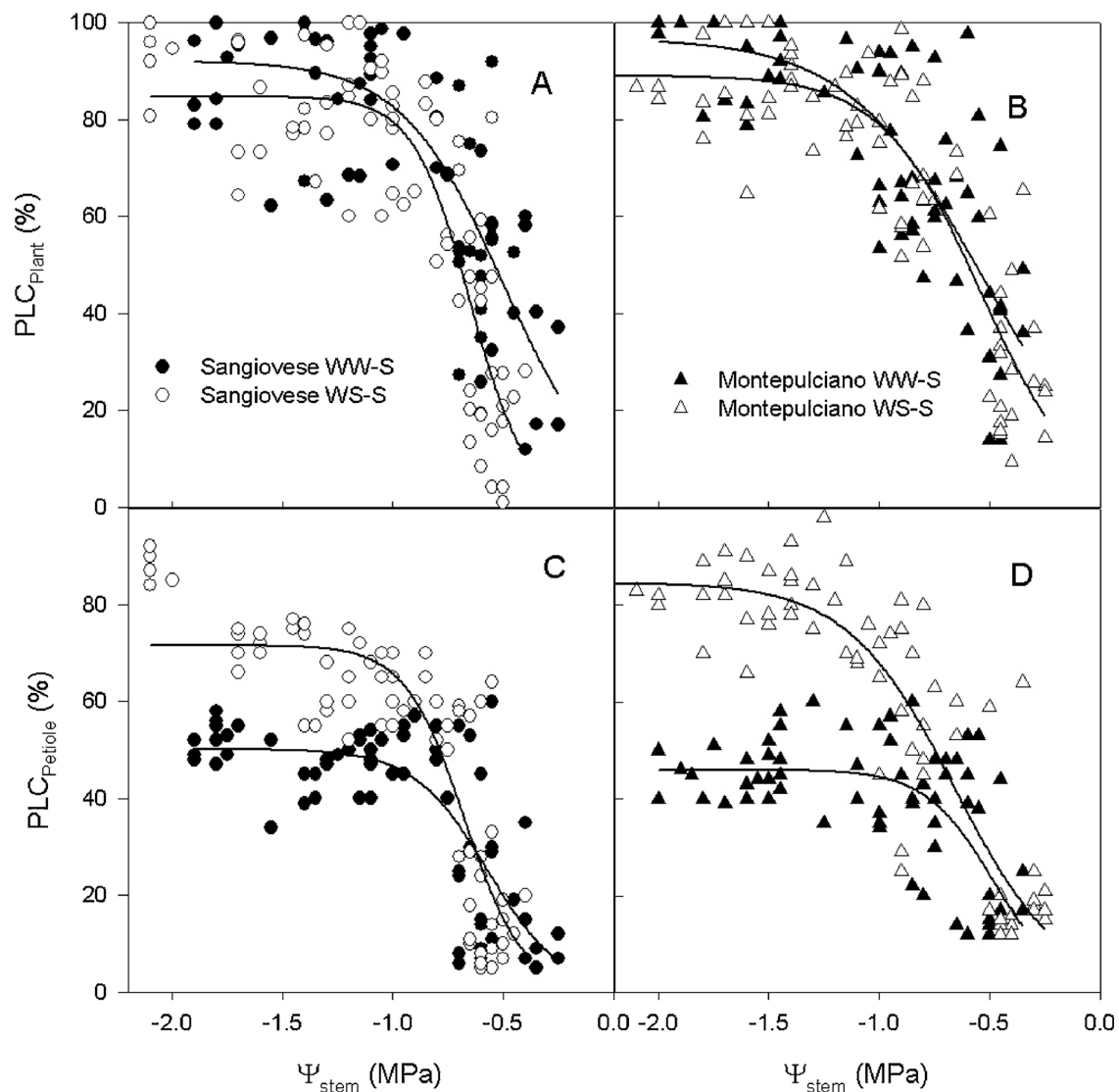
$$PLC_{plant} = 100 \times (K_{plantmax} - K_{plant})/K_{plantmax}$$

where  $K_{plantmax}$  was the plant hydraulic conductance measured on the first day of the experiment when plants were still well irrigated.

Differences between the two treatments were assessed using the Student's *t*-test ( $P < 0.05$ ). The significance of regressions was tested using Pearson Product Moment Correlation.

### 3. Results

Stem water potential at midday ( $\Psi_{stem}$ ) decreased over the experiment with few differences between WS and Control in both cultivars (Fig. 1a,b). However, in few days WS vines had significantly lower  $\Psi_{stem}$  than control vines. Stomatal conductance ( $g_s$ ) approximately followed a similar trend to that of  $\Psi_{stem}$  during the experiment reaching full stomata closure in 9 days (Fig. 1c,d). During this part of the experiment, in both cultivars but especially in Montepulciano,  $g_s$  was consistently higher in WS than in control vines. Net assimilation followed a pattern similar to that of  $g_s$  during the experiment (Fig. 1e,f), and there were no significant differences between treatments at any sampling day.



**Fig. 4.**  $\text{PLC}_{\text{plant}}$  vs.  $\Psi_{\text{stem}}$  in Sangiovese (WW-S  $y = 0.92/(1 + \exp(-(x + 0.50)/-0.23))$ ,  $R^2 = 0.61$   $P < 0.0001$ ; WS-S  $y = 0.85/(1 + \exp(-(x + 0.65)/-0.13))$ ,  $R^2 = 0.73$   $P < 0.0001$ ) (A) and Montepulciano (WW-S  $y = 0.96/(1 + \exp(-(x + 0.54)/-0.30))$ ,  $R^2 = 0.65$   $P < 0.0001$ ; WS-S  $y = 0.89/(1 + \exp(-(x + 0.54)/-0.22))$ ,  $R^2 = 0.79$   $P < 0.0001$ ) (B).  $\text{PLC}_{\text{petiole}}$  vs.  $\Psi_{\text{stem}}$  in Sangiovese (WW-S  $y = 0.50/(1 + \exp(-(x + 0.56)/-0.17))$ ,  $R^2 = 0.58$   $P < 0.0001$ ; WS-S  $y = 0.71/(1 + \exp(-(x + 0.67)/-0.13))$ ,  $R^2 = 0.75$   $P < 0.0001$ ) (C) and Montepulciano (WW-S  $y = 0.46/(1 + \exp(-(x + 0.48)/-0.15))$ ,  $R^2 = 0.47$   $P < 0.0001$ ; WS-S  $y = 0.84/(1 + \exp(-(x + 0.66)/-0.24))$ ,  $R^2 = 0.80$   $P < 0.0001$ ) (D).

Leaf transpiration was significantly ( $P < 0.001$ ) correlated with  $\Psi_{\text{stem}}$  in both treatments and in both cultivars (Fig. 2). In particular, in WS vines, leaf transpiration was higher than in control vines for  $\Psi_{\text{stem}} > -1.5$  MPa. The same pattern occurred for  $g_s$  that, in both cultivars, was higher in WS vines than in control vines at any  $\Psi_{\text{stem}}$  value. Noteworthy, in WS vines the  $\Psi_{\text{stem}}$ , at which stomatal conductance approached zero, was slightly shifted toward more negative  $\Psi_{\text{stem}}$  in comparison with control vines. At any  $\Psi_{\text{stem}}$  value, the difference between treatment means was generally larger in Montepulciano than in Sangiovese.

The relationship between  $\text{PLC}_{\text{plant}}$  and  $\Psi_{\text{stem}}$  did not vary between treatments in both cultivars (Fig. 4a,b). Conversely, the relationship between  $\text{PLC}_{\text{petiole}}$  and  $\Psi_{\text{stem}}$  followed a different pattern depending on the treatment: in control vines,  $\text{PLC}_{\text{petiole}}$  increased up to a plateau close to 50% of PLC that was reached at  $\Psi_{\text{stem}}$  around  $-1$  MPa; on the other hand, in WS vines, PLC increased up to values close to 90% over the  $\Psi_{\text{stem}}$  range tested in this experiment (Fig. 4c,d). The  $\text{PLC}_{\text{petiole}}$  trend was slightly different in WS treatment across the two genotypes tested: in Montepulciano,  $\text{PLC}_{\text{petiole}}$  was higher than in Sangiovese at  $\Psi_{\text{stem}}$  lower than  $-1$  MPa. At  $\Psi_{\text{stem}}$  lower than  $-2$  MPa,  $\text{PLC}_{\text{petiole}}$  was similar in the two genotypes.

There was also a significant relationship between  $g_s$  and  $\text{PLC}_{\text{petiole}}$  in both cultivars and in both treatments (Fig. 5). However, in WS vines the relationship between  $g_s$  and  $\text{PLC}_{\text{petiole}}$  was shifted upward, with larger values of  $g_s$  at any  $\text{PLC}_{\text{petiole}}$  level. Stomata closure occurred at consistently larger  $\text{PLC}_{\text{petiole}}$  percentages in WS vines than in control vines.

When regressed over  $\Psi_{\text{stem}}$ , water use efficiency (WUE expressed as  $A_n/g_s$ ) followed a similar pattern across cultivars and treatments (Fig. 6). Notably, in both Sangiovese treatments and in WS-Montepulciano, WUE peaked at approximately  $-0.8$  MPa  $\Psi_{\text{stem}}$ . Moreover, control vines of both cultivars showed consistently greater WUE at any  $\Psi_{\text{stem}}$  value than WS vines. The relationship between  $\text{PLC}_{\text{petiole}}$  and  $\text{PLC}_{\text{plant}}$  followed a different pattern between treatments (Fig. 7): in WS plants of both cultivars, there was a relationship between  $\text{PLC}_{\text{petiole}}$  and  $\text{PLC}_{\text{plant}}$  close to 1:1 with  $\text{PLC}_{\text{plant}}$  percentages always larger than  $\text{PLC}_{\text{petiole}}$ . In control vines,  $\text{PLC}_{\text{petiole}}$  was always significantly smaller than  $\text{PLC}_{\text{plant}}$  and did not reach the 50% threshold at any  $\text{PLC}_{\text{plant}}$  percentage.

#### 4. Discussion

Repeated drought stress influenced plant strategy by changing



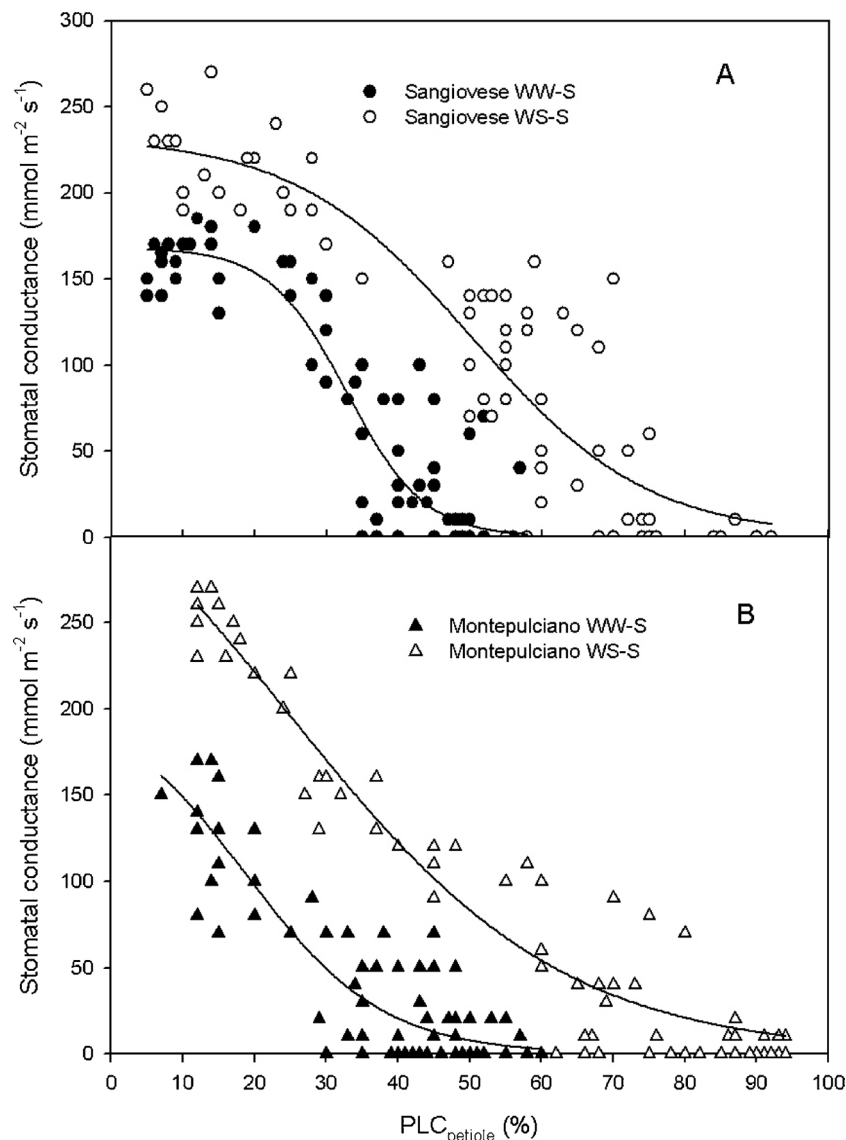
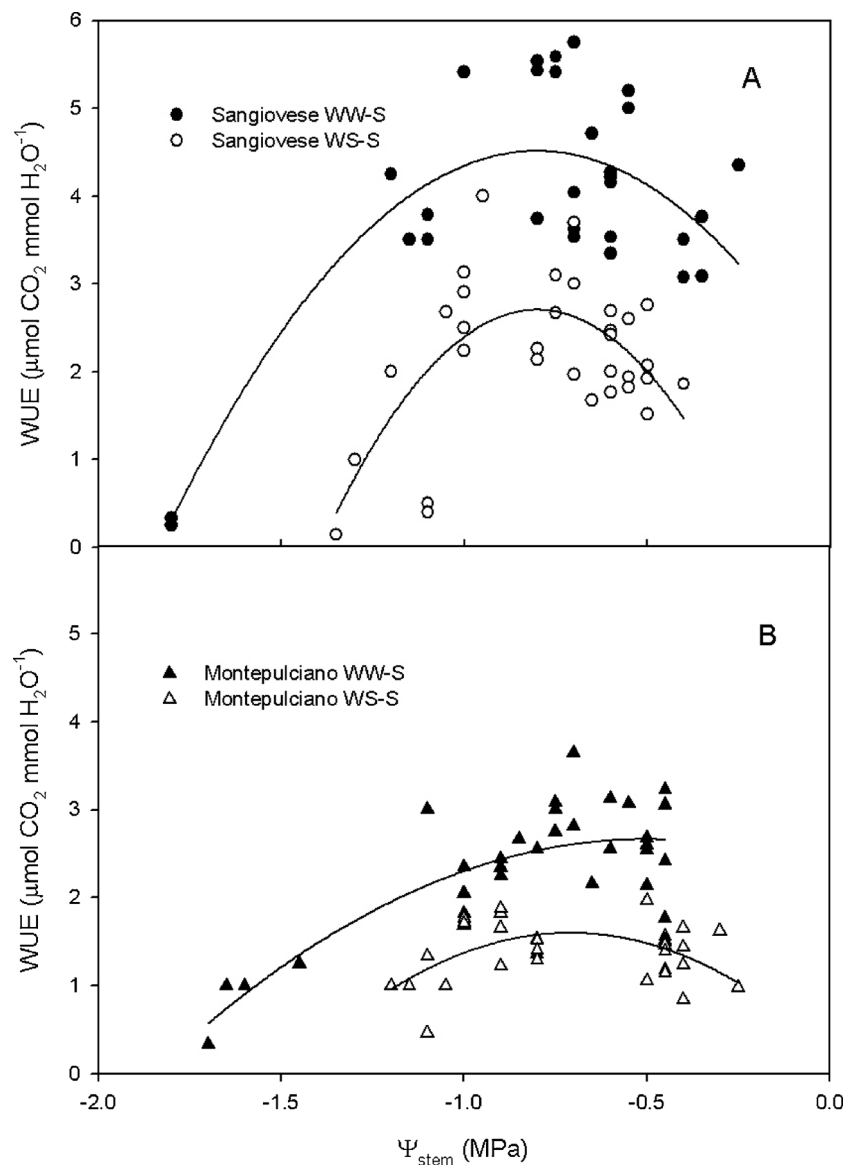


Fig. 5. Stomatal conductance vs. PLC<sub>petiole</sub> in Control and WS vines of Sangiovese (WW-S  $y = 0.17 / (1 + \exp(-(x - 0.33) / -0.05))$ ,  $R^2 = 0.85$   $P < 0.0001$ ; WS-S  $y = 0.23 / (1 + \exp(-(x - 0.50) / -0.12))$ ,  $R^2 = 0.81$   $P < 0.0001$ ) (A) and Montepulciano (B) (WW-S  $y = 0.21 / (1 + \exp(-(x - 0.19) / -0.10))$ ,  $R^2 = 0.80$   $P < 0.0001$ ; WS-S  $y = 0.40 / (1 + \exp(-(x - 0.25) / -0.19))$ ,  $R^2 = 0.94$   $P < 0.0001$ ).

stomatal regulation and hydraulic safety margin. In our experiment, although  $\Psi_{\text{stem}}$  was rather similar across treatments, WS vines of both genotypes had consistently higher stomatal conductance and leaf transpiration during the dry-down phase as compared with Control plants (Figs. 1 and 2). Nonetheless, the  $\Psi_{\text{stem}}$  value inducing the complete loss of stomatal conductance remained almost unchanged in WS-S and WW-S treatment in Sangiovese, but it was slightly lower in WS-S than in WW-S in Montepulciano (Fig. 3). These data clearly indicate that, regardless of the genotype, in WS grapevines stomatal regulation was deeply altered. In previous studies on the grapevine, it has been emphasized the link between stomatal control, that represents the main source of hydraulic resistance in the soil-plant-air continuum (Damour et al., 2010), and xylem vulnerability that appears to influence the stomatal closure dynamics (Tombesi et al., 2014, 2015; Hochberg et al., 2013). Within the plant hydraulic apparatus, leaf petiole has been indicated as the organ most vulnerable to embolism, setting the maximum tension withstood by the plant; in other words, the petiole works such as a hydraulic fuse considering that its xylem vulnerability to embolism is larger than that of stems as measured by Zufferey et al., 2011 and Hochberg et al., 2016. In our experiment, petioles of WS vines reached higher PLC values than those of control plants (Fig. 4C,D). This

could be partly consistent with the cavitation fatigue theory, that postulates that repeated cycles of xylem vessel embolization can cause an increase of the xylem vulnerability (Hacke et al., 2001), although the relationship between PLC<sub>petiole</sub> and  $\Psi_{\text{stem}}$  was similar between treatments for  $\Psi_{\text{stem}} > -1$  MPa. However, different PLC<sub>petiole</sub> sensitivity toward increasing  $\Psi_{\text{stem}}$  in C and WS vines could also be the consequence of different stomatal dynamic rather than increased xylem vulnerability. Indeed, it was surprising to observe this phenomenon on newly formed structures that were not directly exposed to drought events occurring over the 4 previous seasons (i.e. each vine was spur-pruned during winter and only few nodes of the previous year vegetation were retained). Even more surprising was the different relationship between stomatal conductance and leaf petiole vulnerability: stomata in WS vines closed at far larger values of petiole PLC, as compared with control vines. This suggests a plant adjustment that may allow decreasing stomatal limitation of photosynthesis at sub-optimal water potential values.

However, it should be noted that in our experiment, throughout the whole dry-down period, net assimilation rates were similar across treatments, and photosystem II efficiency, evaluated through the Fv/Fm parameter, was similar between WS and control plants (data not



**Fig. 6.** Water use efficiency (WUE) vs.  $\Psi_{\text{stem}}$  in Control and WS vines of Sangiovese (Control  $y = 1.79 - 6.77x + -4.22x^2$ ,  $R^2 = 0.65$   $P < 0.0001$ ; WS  $y = -2.23 - 12.32x + -7.68x^2$ ,  $R^2 = 0.40$   $P = 0.0005$ ) (A) and Montepulciano (B) (Control  $y = 2.29 - 1.47x + -1.47x^2$ ,  $R^2 = 0.59$   $P < 0.0001$ ; WS  $y = 0.24 - 3.80x + -2.68x^2$ ,  $R^2 = 0.22$   $P = 0.026$ ).

shown). In fact, the increase in leaf transpiration and the concurrent lack of increase in photosynthetic activity caused the reduction of WUE in WS plants at any level of water stress.

In general, drought tolerance is related to the capability of keeping a conservative strategy toward water loss (Levitt, 1980). During water stress stomata down-regulate water loss to protect xylem apparatus and, within certain water potential intervals, this might cause an increase in the water use efficiency (Chaves et al., 2010). This is consistent with our experiment in which WUE increased for the intervals between  $-0.3$  and  $-0.8$  MPa and between  $-0.3$  and  $-0.7$  MPa in Sangiovese and Montepulciano, respectively (Fig. 6). In this range water potential was not limiting for plant assimilation, but water availability was gradually approaching the  $\Psi_{\text{stem}}$  at which stomatal conductance becomes affected by water potential. Nonetheless, WS plants, that should be more adapted to drought theoretically, exhibited, at  $\Psi_{\text{stem}} < -0.7$ – $-0.8$  MPa, a generally lower WUE in comparison with control vines due to higher transpiration rates (Merli et al., 2015). Such unexpected results indicate that, in perennials, such as grapevine, drought stress in previous years could cause a change in the regulation of transpiration opposite to that (reduction of transpiration and increase in WUE) generally described as

a drought avoidance behavior (Levitt, 1980). The increase in leaf transpiration observed in our experiment was similar to that observed on vines double stressed on the same season (i.e. vines were subjected to two water stress periods over the same season) when these were compared with the behavior of vines subjected to a single water stress after being well irrigated during the previous part of the season (Hochberg et al., 2017). These results contradict the postulation that plants adapt their physiological behavior to become more conservative in terms of water use, hence to avoid water stress (Schwinning and Ehleringer, 2001). In this experiment previous stress did not make plant more tolerant to stress and in this specific case to water stress as generally assumed (Bruce et al., 2007; Li and Liu, 2016; Fleta-Soriano and Munné-Bosch, 2016). WUE decreased in WS-S plants in comparison with WW-S, furthermore thought in WS-S stomatal conductance was down regulated at  $\Psi_{\text{stem}}$  values lower than in WW-S and transpiration was larger in WS-S potentially leading to a faster depletion of water stock in the soil during prolonged drought. However, these data may suggest alternative, non conservative strategies used by plants to exploit available water under recurrent drought. In a recent study on 32 gymnosperm and 192 angiosperm species, the minimum  $\Psi_{\text{stem}}$  was

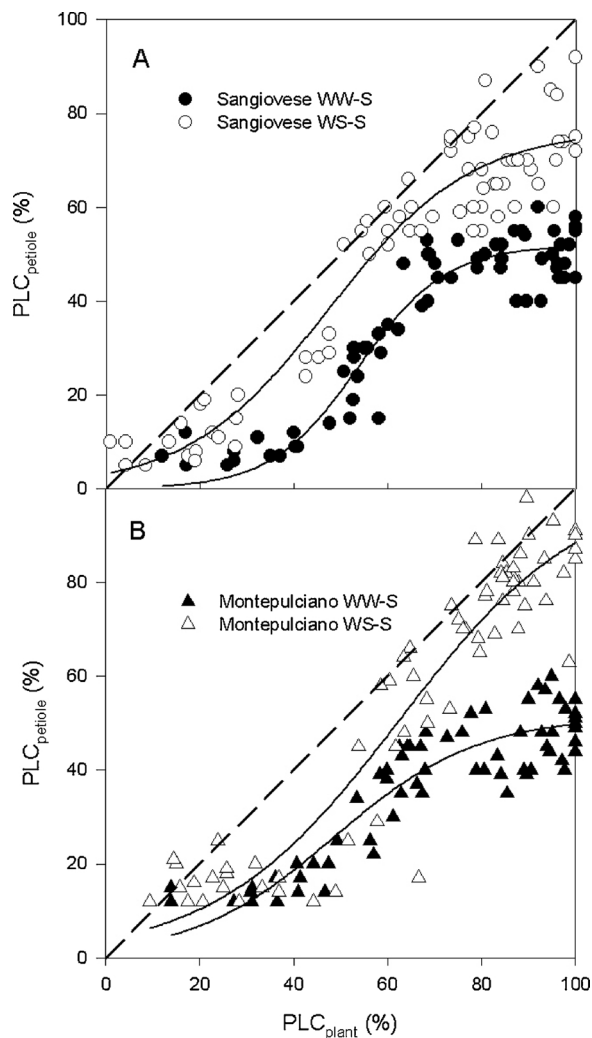


Fig. 7.  $PLC_{petiole}$  vs  $PLC_{plant}$  in Control and WS vines of Sangiovese (WW-S  $y = 0.52 / (1 + \exp(-(x - 0.54)/0.09))$ ,  $R^2 = 0.91$   $P < 0.0001$ ; WS  $y = 0.77 / (1 + \exp(-(x - 0.47)/0.15))$ ,  $R^2 = 0.92$   $P < 0.0001$ ) (A) and Montepulciano (B) (Control  $y = 0.51 / (1 + \exp(-(x - 0.48)/0.15))$ ,  $R^2 = 0.83$   $P < 0.0001$ ; WS  $y = 1.01 / (1 + \exp(-(x + 0.62)/0.2))$ ,  $R^2 = 0.88$   $P < 0.0001$ ).

significantly correlated with the  $\Psi_{stem}$  at which 50% of loss of hydraulic conductance occurs (Choat et al., 2012). In particular, in angiosperm species, minimum  $\Psi_{stem}$  was quite close to  $\Psi_{50}$  indicating a narrow safety margin (i.e. differences between naturally occurring xylem pressures and pressures that would cause hydraulic dysfunction) (Johnson et al., 2012). As far as we know, our study points out for the first time the capacity of plants, in this specific case *V. vinifera*, to decrease their safety margin when exposed to recurrent water stress condition. In fact, the looser control on leaf transpiration rates at limiting water potential let the vines operate at higher  $PLC_{petiole}$  level than in vines that never experienced water stress, whose  $PLC_{petiole}$  never exceeded the 50% threshold. This resulted in a larger capability of transpiring under water stress condition and, concurrently, it adjusted plant hydraulic conductance to the limit of leaf petiole vulnerability. Such strategy could be evaluated as positive because it reduces the limitation of transpiration and this could indirectly decrease the long term damages associated with the loss of energy dissipation via transpiration. At the same time such strategy may expose plant to a quick depletion of soil water reservoir and to larger damages in peripheral organs if water availability further decreases; smaller safety margins in previously stressed plants may cause an increase in runaway embolism phenomena in petioles and the loss of a large part of leaf area. However, it should be considered that *V. vinifera* is a deciduous perennial, and

that leaf shedding is a common strategy to decrease plant transpiration and to overcome intense drought periods (Bréda et al., 2006). Thus, the loss of leaves does not compromise plant survival (at the end of the experiment vines shed almost all leaves but after the experiment, after rewatering, dormant buds sprouted and formed new leaves). On the other hand, a larger capability of transpiring under water stress condition allows plant to operate at generally lower water potentials reducing limitations due to reduction of transpiration induced by water stress.

Increased capability to transpire could be compatible with the increase in root biomass that is a common long-term adaptation to xeric environment (De Micco and Aronne, 2012). Unfortunately, in this experiment it was not possible to test if there was any coordination between these two traits.

## 5. Conclusions

Water stress in the previous seasons caused an increase in  $PLC_{petiole}$  over current season water stress in comparison with plants that did not experience water stress in the previous seasons. This was caused by a different regulation of transpiration by stomata that let the plant transpire more water during plant water potential drop caused by the progressive reduction of water in the soil. This caused a decrease in WUE but, on the other hand, a larger capability to keep leaf active at relatively low water potential. These results indicate that plant acclimation to recurrent drought induce a reduction of the safety margin at which plants operate. This mechanism allows plants to withstand larger soil water content fluctuations at the cost of higher risk for peripheral organs such as petioles and, consequently, leaves.

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## References

- Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree population. *Evol. Appl.* 1, 95–111.
- Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann. For. Sci.* 63, 625–644.
- Bruce, T.J.A., Matthes, M.C., Napier, J.A., Pickett, J.A., 2007. Stressful memories of plants: evidence and possible mechanisms. *Plant Sci.* 173, 603–608.
- Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Arvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field? Photosynthesis and growth. *Ann. Bot.* 89, 907–916. <http://dx.doi.org/10.1093/aob/mcf105>.
- Chaves, M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues, M.L., Lopes, C.M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.* 105, 661–676.
- Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Feild, T.S., Gleason, S.M., Hacke, U.G., et al., 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491, 752–755.
- Dai, A., 2010. Drought under global warming: a review. *Wiley Interdiscip. Rev. Clim. Change* 2, 45–65.
- Damour, G., Simonneau, T., Cochard, H., Urban, L., 2010. An overview of models of stomatal conductance at the leaf level. *Plant Cell Environ.* 33, 1419–1438.
- Daszkowska-Golec, A., Szarejko, I., 2013. Open or close the gate-stomata action under the control of phytohormones in drought stress conditions. *Front. Plant Sci.* 4, 138. <http://dx.doi.org/10.3389/fpls.2013.00138>.
- De Micco, V., Aronne, G., 2012. Morpho-anatomical traits for plant adaptation to drought. In: Arco, R. (Ed.), *Plant Responses to Drought Stress*. Springer-Verlag, Berlin Heidelberg, pp. 37–61.
- FAOSTAT (2018). <http://www.fao.org/faostat>, Accessed on March 2018.
- Fleta-Soriano, E., Munné-Bosch, S., 2016. Stress memory and the inevitable effects of drought: a physiological perspective. *Front. Plant Sci.* 7, 143.
- Franks, S.J., Weber, J.J., Aitken, S.N., 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evol. Appl.* 7, 123–139.
- Franks, P.J., 2004. Stomatal control and hydraulic conductance, with special reference to tall trees. *Tree Physiol.* 24, 865–878.



- Glenn, D.M., Kim, S.H., Ramirez-Villegas, J., Laderach, P., 2014. Response of perennial Horticultural crops to climate change. *Hortic. Rev.* 41, 47–130.
- Grayson, M., 2013. Agriculture and drought. *Nature* 501 (S1), 10.
- Hacke, U.G., Stiller, V., Sperry, J.S., Pittermann, J., McCulloh, K.A., 2001. Cavitation fatigue: embolism and refilling cycles can weaken the cavitation resistance of xylem. *Plant Physiol.* 125, 779–786.
- Hochberg, U., Degu, A., Fait, A., Rachmilevitch, S., 2013. Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. *Physiol. Plant.* 147, 443–452.
- Hochberg, U., Albuquerque, C., Rachmilevitch, S., Cochard, H., David-Schwartz, R., Brodersen, C.R., McElrone, A., Windt, C.W., 2016. Grapevine petioles are more sensitive to drought induced embolism than stems: evidence from in vivo MRI and microCT observations of hydraulic vulnerability segmentation. *Plant Cell Environ.* 39, 1886–1894.
- Hochberg, U., Bonel, A.G., David-Schwartz, R., Degu, A., Fait, A., Cochard, H., Herrera, J.C., 2017. Grapevine acclimation to water deficit: the adjustment of stomatal and hydraulic conductance differs from petiole embolism vulnerability. *Planta* 245, 1091–1104.
- Johnson, D.M., McCulloh, K.A., Woodruff, D.R., Meinzer, F.C., 2012. Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Sci.* 195, 48–53.
- Jones, H.G., Sutherland, R.A., 1991. Stomatal control of xylem embolism. *Plant Cell Environ.* 11, 111–121.
- Levitt, J., 1980. Responses of plants to environmental stresses. *Physiological Ecology Series*. Academic Press, Michigan, USA.
- Li, X., Liu, F., et al., 2016. Drought stress Memory and Drought stress tolerance in plants: biochemical and molecular basis. In: In: Hossain, M.A. (Ed.), *Drought Stress Tolerance in Plants 1*. Springer International Publishing, Switzerland, pp. 17–44.
- Lovisolo, C., Schubert, A., 1998. Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *J. Exp. Bot.* 49, 693–700.
- Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., Schubert, A., 2010. Drought induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole plant level: a physiological and molecular update. *Funct. Plant Biol.* 37, 98–116.
- McCutchan, H., Shackel, K.A., 1992. Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. cv. French). *J. Am. Soc. Hortic. Sci.* 117, 607–611.
- Merli, M.C., Gatti, M., Galbignani, M., Bernizzoni, F., Magnanini, E., Poni, S., 2015. Water use efficiency in sangiovese grapes (*Vitis vinifera* L.) subjected to water stress before veraison: different levels of assessment lead to different conclusions. *Funct. Plant Biol.* 42, 198–208.
- Moriondo, M., Jones, G.V., Bois, B., Dibari, C., Ferrise, R., Trombi, G., Bindi, M., 2013. Projected shifts of wine regions in response to climate change. *Clim. Change* 119, 825–839.
- Nardini, A., Salleo, S., 2000. Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees* 15, 14–24.
- Palliotti, A., Tombesi, S., Frioni, T., Famiani, F., Silvestroni, O., Zamboni, M., Poni, S., 2014. Morpho-structural and physiological response of container-grown sangiovese and Montepulciano cvv. (*Vitis vinifera*) to re-watering after a pre-veraison limiting water deficit. *Funct. Plant Biol.* 41, 634–647.
- Salleo, S., Nardini, A., Pitt, F., Lo Gullo, M.A., 2000. Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.). *Plant Cell Environ.* 23, 71–79.
- Sarwat, M., Tuteja, N., 2017. Hormonal signaling to control stomatal movement during drought stress. *Plant Gene* 11 (part B), 143–153.
- Schwinning, S., Ehleringer, J.R., 2001. Water use trade-offs and optimal adaptations to pulse-driven arid ecosystems. *J. Ecol.* 89, 464–480.
- Sheffield, J., Wood, E.F., 2008. Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations. *Clim. Dyn.* 13, 79–105.
- Sheffield, J., Wood, E.F., Roderick, M.L., 2012. Little change in global drought over the past 60 years. *Nature* 491, 435–438.
- Sperry, J.S., Pockman, W.T., 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant Cell Environ.* 16, 279–287.
- Tombesi, S., Nardini, A., Farinelli, D., Palliotti, A., 2014. Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of *Vitis vinifera*. *Physiol. Plant.* 152, 453–464.
- Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni, S., Palliotti, A., 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. *Sci. Rep.* 5, 12449.
- Trenberth, K.E., Dai, A., Schrier van der, G., Jones, P.D., Barichivich, J., Briffa, K.R., Sheffield, J., 2014. Global warming and changes in drought. *Nat. Clim. Change* 4, 17–22.
- Trifilò, P., Casolo, V., Raimondo, F., Petrusa, E., Boscutti, F., Lo Gullo, M.A., Nardini, A., 2017. Effects of prolonged drought on stem non-structural carbohydrates content and post-drought hydraulic recovery in *Laurus nobilis* L.: the possible link between carbon starvation and hydraulic failure. *Plant Physiol. Biochem.* 120, 232–241.
- Tuteja, N., Sopory, S.K., 2008. Chemical signaling under abiotic stress environment in plants. *Plant Signal. Behav.* 3, 525–536.
- Tyree, M.T., Sperry, J.S., 1989. Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 19–38.
- Wilhite, D.A., 2000. Drought as a natural hazard: concepts and definitions. In: In: Wilhite, D.A. (Ed.), *Drought: A Global Assessment 1*. Routledge, London, pp. 3–18.
- Zufferey, V., Cochard, H., Ameglio, T., JI, S., Viret, O., 2011. Diurnal cycles of embolism formation and repair in petioles of grapevine (*Vitis vinifera* cv. Chasselas). *J. Exp. Bot.* 62, 3885–3894.